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BACTERIOLOGICAL EXAMINATIONS OF SWIMMING POOLS IN MANILA [1]

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(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

ONE TEXT FIGURE

INTRODUCTION

While it is true that disease is not frequently acquired as a result of infection while bathing, it is of paramount importance that the water used for bathing be free from infectious material. Bacteria such as *Bacillus coli, B. typhosus, B. diphtheriæ, B. dysenteriæ, Spirillum choleræ, Micrococcus gonorrheæ,* and the various pathogenic organisms found in superficial lesions or occasionally on the normal skin may be disseminated by this means, as may be intestinal parasites belonging to the animal kingdom. Bathers who fail to cleanse themselves properly before entering a pool or those who are carriers and hence disseminators of pathogenic organisms may contaminate the water in the tank.

It has been demonstrated on numerous occasions not only that water has thus been contaminated, but also that bathers have been infected.[2]

Bacterial examinations made of bath water in the prison at Kyoto [3] showed the presence of *B. tuberculosis, B. tetani,* gono-

[1] Received for publication March 15, 1916.

[2] Skutsch, *Centralbl. f. Bakt.* etc. (1892), 12, 309; Jäger, *Zeitschr. f. Hyg. u. Infectionskrankh.* (1892), 12, 525; Schultz, *Berl. klin. Wochenschr.* (1899), 36, 865; Fehr, Ibid. (1900), 37, 10.

[3] Nakao Abe, *Arch. f. Hyg.* (1908), 65, 140.

coccus, and pneumococcus. The fresh water in the tub (about 160 liters) showed 1,500,000 bacteria per cubic centimeter; after one person bathed in it, the bacterial content was 5,300,000; and after twenty persons used it, 2,086,600,000 bacteria per cubic centimeter were found. In another tank of about 4,800 liters the unused water had 700,000 bacteria per cubic centimeter; after two hundred persons had bathed in the tank, the water had 20,400,000 bacteria per cubic centimeter; after six hundred persons had bathed in it, there were 683,000,000 bacteria per cubic centimeter; and after nine hundred persons had bathed in it, there were 1,799,000,000 bacteria per cubic centimeter.

A series of tests on the waters of certain swimming pools in Germany gave the following results:[4]

POOL A.

Test No.	Date.	Bacteria per cc.
1	Jan. 6, 1893	9,700
2	Jan. 18, 1893	50,000
3	Jan. 25, 1893	75,000

The water of this pool was renewed each day and kept at a temperature of 22°C. Samples for examination were taken from the corner of the basin. In the center of the pool the bacterial content was somewhat greater.

Pool B had 400 cubic meters of water. It was refilled every second day after being well cleaned and was kept at a temperature of 20.6°C. February 2, at 1 o'clock in the afternoon, the water contained 53,500 bacteria per cubic centimeter, after being in the pool for forty hours. February 8, the water contained 22,000 bacteria per cubic centimeter, after being in the pool for fifteen hours.

Pool C contained 180 cubic meters of water. On March 9, at 9 o'clock in the morning, the bacterial content of the pool was 23,000 per cubic centimeter; in the center it was 41,500 per cubic centimeter. On March 16, at 6 o'clock in the afternoon, the bacterial content in the corner of the pool was 6,800 per cubic centimeter; in the center it was 14,000 per cubic centimeter.

Bacteriological examinations of swimming pools in the United States have been comparatively recent and few. Only in comparatively recent years have municipalities established and main-

[4] Max Edel, *Arch. f. Hyg.* (1893), 19, 233.

tained public natatoria, their cost[5] having probably hindered their establishment in larger numbers. Pools maintained by educational institutions have been examined more carefully than the majority of public pools, since the facilities for bacteriological work were near at hand.[6]

The occasional established instances of public-bath infections, together with exaggerated popular accounts of acquired sores, ear troubles, venereal diseases, etc., alleged to be due to infection from contaminated swimming tanks, have caused many to look with aversion upon water that has been in contact with other persons. Since contagious diseases are more prevalent in the tropics than in temperate regions, it is especially important that in countries like the Philippine Islands every possible precaution be taken to prevent the dissemination of infectious material through the medium of bath waters. Much more work has been done on the purification of sewage and of drinking water than of bath water, but fortunately the results of the work on the former are in a great measure applicable to the latter.

Both physical and chemical methods of purification have been employed in attempts to render such waters innocuous. Among the physical methods that have been used to purify water bacteriologically are heat, ultra-violet light, and filtration. These methods have generally been considered too costly for swimming pools. The last gives good results and is used to some extent, but not nearly as much as for drinking water. Heating water to from 60° to 70°C. has been found[7] to give adequate purification.

Many chemical methods have been devised for purifying water. Certain of these methods, through the coagulation of colloids and the formation of precipitates, lessen the turbidity of the water, causing it to appear fresher and cleaner. The use of such substances as mineral acids, alkalies, potassium permanganate, corrosive sublimate, sodium benzoate, boric acid, and ozone has been recommended, but most of them have proved to be too expensive for general use or otherwise im-

[5] Report of the President of the Borough of Manhattan, New York City (1912).

[6] Bunker, J. W. M., *Science* (1910), n. s. 31, 556; Ravenel, *Am. Phy. Ed. Rev.* (1912), 17, 684; Manheimer, W. A., *Pub. Health Rep.* (1915), 30, 2796.

[7] Dunbar, W. P., Leitfaden f. d. Abwasserreinigungsfrage. R. Oldenbourg, Berlin (1907), 341.

practical. In practice one of the first substances to be used was quicklime. However, a large amount must be added in order to obtain efficient disinfection, and it often happens that sufficient precipitate is formed greatly to increase the turbidity of the water. Copper sulphate has had various advocates,[8] but while it is very useful when used against plant contaminations such as algæ, it cannot take front rank so far as its bactericidal results are concerned. It, too, has the disadvantage of greatly increasing the turbidity of certain waters.

Chlorine and compounds like hypochlorites, from which chlorine is readily liberated, are comparatively cheap and are much used. They are thought to owe their germicidal action to the liberation of oxygen, thus acting like ozone. They seldom produce undesirable precipitates and have little toxicity. Antiformin has been recommended as a disinfectant by Uhlenhuth and Xylander.[9] Phelps [10] found that the chlorine was not liberated as rapidly from it as from hypochlorite of calcium, but that its use was not more efficient in reducing bacterial content.

Hypochlorite of calcium still seems to be, according to many investigators, the most generally useful disinfectant for sewage and for potable and bath water. Manheimer [11] recommends its use, testing the water from time to time in order to determine that a trace of chlorine is kept constantly present. Bunker [12] found that the addition of bleaching powder in quantities sufficient to give one part available chlorine in 2,000,000 parts of water kept the water sterile for four days with constant use of the pool. He recommended the application of the disinfectant twice a week for the average pool, to insure practically sterile water.

At the University of Wisconsin tank, which has a capacity of 97,000 gallons, Ravenel [13] found that there was a gradual increase in the number of bacteria to the middle of the week, then a decrease, but another increase on Saturday. Treating 250 cubic centimeter samples of water with calcium hypochlorite for thirty minutes, it was found that about 0.5 part of available chlorine per million parts of water materially reduced the bacterial content and usually destroyed the colon bacilli. One

[8] Stokes, *Am. Med.* (1905), 10, 1075; Burrage, *Proc. Ind. Assoc. Sci.* (1909); Rettger and Markey, *Eng. News* (1911), 66, 699.

[9] *Berl. klin. Wochenschr.* (1908), 46, 1346.

[10] Phelps, E. B., Gesundheits-Ingenieur. Anklam, Munich (1910), 407.

[11] *Pub. Health Rep.* (1915), 30, 2796.

[12] *Science* (1910), n. s. 31, 556.

[13] *Am. Phy. Ed. Rev.* (1912), 17, 684.

part per million made the water practically sterile. It appeared that the effect of the hypochlorite lasted only about three days, after which there was a considerable increase in bacteria.

When unchlorinated, the water at the University of Pennsylvania showed a continued increase in numbers of bacteria.[14] The tank has a capacity of 155,000 gallons, and city water was used. After adding alum as a coagulant, the water was filtered before it entered the tank. Fifteen hundred gallons were allowed to flow through the tank daily. Every Sunday the tank was emptied, scrubbed, and refilled. *Bacillus pyocyaneus* was frequently isolated from the water. Part of the results obtained is appended herewith.

Bacteria per cubic centimeter found in University of Pennsylvania swimming tank.

[Grown 24 hours on agar.]

BACTERIA IN UNCHLORINATED WATER.

Day.	Colonies per cc.
1	85
2	340
3	428
4	3,000
5	7,090
6	50,000

On Thursday approximately 0.5 part of available chlorine per million parts of water was added to the water, giving the following results:

BACTERIA IN CHLORINATED WATER.

	Colonies per cc.
Before adding chlorine	50,000
15 minutes after adding chlorine	250
2 hours after adding chlorine	0
24 hours after adding chlorine	160

Some of the conclusions drawn from the work just mentioned were that pathogenic organisms may readily find entrance to the water and possibly cause disease; that small amounts of chlorine added to the water in the tank every morning quickly destroy the microörganisms present; that accumulations of hair and other débris at the bottom of the tank should be removed daily by small hand pumps, as they are less readily disinfected than the water.

There are many factors influencing the sterilizing action of chlorine and hypochlorites on water, and a great part of the conflicting evidence found in the literature relative to quantity

[14] W. J. Lyster, *Journ. Am. Med. Assoc.* (1911), 57, 1992.

of disinfectant necessary for efficient purification, time required for sterilization, or duration of bactericidal effect is due to the influence of one or more of these factors. Quality of water, the amount of disinfectant, decomposition rate of the disinfectant, temperature, illumination of the pool, period of time water was in use, extent to which bacteria have gravitated to the bottom, manner of cleaning tank, wind, and other factors modify the biological conditions. Heise [15] has shown that an addition of calcium hypochlorite equivalent to 1 part of available chlorine per million parts of the water used in the Manila swimming pools is reduced in two hours to about 0.1 part.

The bactericidal value of chlorine is reduced [16] by the chlorine-consuming power of various substances with which it may come in contact. Thus Heise [17] found that on adding bleaching powder equivalent to 32 parts of chlorine per million parts of water and allowing it to act two hours in diffused daylight at 28°C. the amount consumed was:

	Parts.
In distilled water	0.75
In city water (unchlorinated)	1 to 2.5
In 200 cc. distilled water +0.5 cc. saliva	10.0
In 200 cc. distilled water +0.5 cc. sweat	28.0
In 200 cc. distilled water +1.0 cc. urine	23.5

The preceding data show that the contamination of a pool by substances such as the secretions and excretions of the human body should be avoided so far as possible, not only because such materials carry bacterial flora with them and in themselves often furnish food material for microöganisms, but also because they actually reduce the amount of available chlorine.

POOLS TESTED IN PRESENT WORK

Tests of the bacterial condition of swimming pools have usually been made in temperate zones. On account of the special conditions met with in the tropics, it seemed advisable to make tests on the three swimming pools of Manila. These pools will be designated by the numerals I, II, and III. Pools I and II were made of concrete and were inclosed on the sides. Pool III was lined with glazed tile and was protected by a roof, but was not inclosed on the sides. The size of these tanks is shown in Table A.

[15] *This Journal, Sec. A* (1916), 11, 112.
[16] Glaser, *Arch. f. Hyg.* (1913), 77, 165.
[17] *This Journal, Sec. A* (1916), 11, 114.

TABLE A.—*Approximate dimensions of the swimming pools tested in Manila.*

Pool.	Length.		Width.		Depth.		Capacity.	
	Meters.	Feet.	Meters.	Feet.	Meters.	Feet.	Cubic meters.	Gallons.
I	18.3	60	6.0	20	1.2 to 2.4	4 to 8	200	52,400
II	18.3	60	5.5	18	1.2 to 2.4	4 to 8	200	52,400
III	18.3	60	7.2	23.5	1.2 to 2.7	4 to 9	225	59,400

The city water supply was used by all. This is a river water, which is chlorinated by adding about 0.5 part of available chlorine per million parts of water after passing through a reservoir. The temperatures of the pools were reasonably constant during the period of examination, varying only from one to two degrees from 28.5°C. The water was changed each week on Sunday, except during the week of November 8 to 13, when the water of the previous week continued in use. The disinfectant was applied by first dissolving it in a pail of water and then scattering the solution over the water in the pool. When the bleaching powder was thus properly distributed, no irritant effect on the eyes of bathers or other objectionable features to its use were detected. At pools I and II it was customary simply to drain off the old water and put in the new, while at pool III the emptied tank was thoroughly cleaned before being refilled. The advantage of the latter process in reducing the original bacterial count is strikingly shown in Table I, August 30, where the counts of the uncleaned pools after refilling averaged 68,000 bacteria per cubic centimeter as against 3,600 for the cleaned pool.

METHODS USED AND RESULTS OBTAINED

The one hundred eighty-nine samples of the water tested were collected in 50 cubic centimeter, sterile, cotton-stoppered bottles at about 8 o'clock in the morning. At the pool a sample bottle was fastened to a stick, the plug was signed and removed, and the specimen of water was obtained by thrusting the bottle about 1 meter below the surface. These bottles were carried in a covered metallic box, to protect them from dust, rain, and sunlight. The samples were usually plated within forty minutes of the time they were taken.

In this work, unless otherwise stated, the methods and recommendations of the committee on standard methods of water analysis of the American Public Health Association (1912) were

followed, except that meat extract was used in making the media. The fermentation tubes (bouillon or bile) contained 1 per cent of lactose. No peptone was added to the bile. Plates were counted and production of gas was determined after twenty-four hours' incubation. When the presumptive test for *Bacillus coli* was positive, 1 cubic centimeter of the medium in the fermentation tube was plated, lactose litmus agar and Endo medium being used for the confirmatory tests.

Besides these media, which are customarily used to show acid and gas producers, Congo red [18] agar was also tried. This shows the presence of *coli*-like organisms by a darkening of the medium, the acid produced causing a dark blue or black precipitate, which can be more readily seen than the changes of color on lactose litmus agar plates.

This medium was found at times to give positive results when litmus lactose agar did not show the presence of *B. coli*, although the fermentation tube and the subsequent attempts to isolate *B. coli* on litmus lactose agar were positive. Although, as was to be expected, litmus lactose agar plates gave smaller counts at 37°C. than agar at 25°C., Congo red agar at 37°C. gave the highest counts. For the confirmatory tests for *B. coli*, Endo medium seemed to be more suitable than Congo red or litmus lactose agar.

In making the presumptive test for *B. coli*, the bile medium used was found less efficient than lactose bouillon or neutral red lactose bouillon. The fermentation tubes, into which 10 cubic centimeters of the water to be tested were inoculated, contained 30 cubic centimeters of the medium. The results of these tests are summarized in Table XIII.

TESTS MADE AND DISCUSSION OF RESULTS

Before trying to determine the effect of disinfectants on the swimming-pool waters, preliminary tests were made to get an idea of the biological condition of the pools and of the variations which occur during use. The results obtained showed that the bacterial content of the swimming pools was not excessive. The plates incubated at blood heat showed a lower count than those kept at the temperature of the room (29°C.). The average count for pool I was highest; this pool had been used by the largest number of persons.

As the tanks were used most extensively in the afternoon, it

[18] Liebermann, L., and Acél, J., *Deutsche Med. Wochenschr.* (1914), 51, 2093.

was thought advisable to determine the variations in bacterial count between the water collected in the morning and that collected in the afternoon.

TABLE B.—*Comparison of morning and afternoon bacterial content of swimming pools.*

[Numbers indicate colonies per cubic centimeter of water.]

Sample taken—	Pool I.				Pool II.			
	Plain agar.	Litmus lactose agar.	Congo red agar.	Average.	Plain agar.	Litmus lactose agar.	Congo red agar.	Average.
Aug. 31 at 8 a. m	2,000	2,500	1,400	2,000	4,800	4,700	3,300	4,300
Aug. 31 at 3 p. m	4,000	10,000	1,800	5,300	40,000	23,000	13,000	25,500
Sept. 4 at 7.30 a. m	100	4,300	2,100	2,200	600	3,500	4,100	2,700
Sept. 4 at 4.10 p. m	3,600	200	1,900	1,900	4,200	4,100	2,200	3,500

From the data in Table B it is seen that the bacterial count in the afternoon is usually higher than early in the morning, when the water has not been stirred up so much by swimmers. The average forenoon count for pools I and II was 2,800 and the average afternoon count was 9,050. For pool III the difference was even greater, the forenoon count being 2,300 and the afternoon count averaging 66,700. A study of the attendance at these pools showed that neither the weekly nor daily variations were sufficiently great seriously to interfere with our work and results. The daily general average of attendance was 32.

The average counts of the three pools on all samples taken before September 20 are given here to exemplify the variations due to different media at the temperatures used.

Average counts on samples from three pools.

	Average number of colonies.
Plain nutrient agar, 25° C.	9,500
Litmus lactose agar, 37° C.	9,100
Congo red agar, 37° C.	11,600

The average counts of all samples taken after September 20 on plain agar are 462,290 for 25°C. and 537,445 for 37°C. incubation, the former being about 88 per cent of the latter.

In a number of instances, after samples had been removed for bacteriological tests, a few cubic centimeters of bouillon were added to the bottle of water in order to promote the multiplication of protozoa which might be present. These specimens were examined microscopically, after three days' storage at room temperature.

Similar tests were made on untreated water from the reservoir, and on the chlorinated tap water as drawn at the laboratory, which probably corresponded to the water used in the swimming pools. The results appear in Table C.

TABLE C.—*Protozoa found in water.*

[A, amœbæ; C, ciliates; F, flagellates.]

Date.	Pool—			Reservoir.	Tap.
	I.	II.	III.		
1915.					
Aug. 30	F	F	F	F	ACF
Aug. 31	AF	C	A	F	AF
Sept. 1	A	AF	AC	Negative	A
Sept. 2	A	A	A	F	A
Sept. 3	F	C	A	Negative	AF
Sept. 4	F	A	.....	A	AF
Sept. 6	F	F	.....	Negative	AF
Sept. 7	Negative	Negative	.....	Negative	C
Sept. 8	F	AC	.....	Negative	Negative
Sept. 9	A	AC	.....	F	F
Sept. 10	AC	A	.....	Negative	F
Sept. 11	F	AF	.....	AF	F

These findings seemed to indicate that the swimming-pool waters were no more contaminated with protozoa than the city water supply. As there is not sufficient evidence to show that these are any but harmless organisms, the determinations along this line were discontinued.

In order to ascertain the extent of pollution of the pool, tests for *Bacillus coli* were made for eleven weeks both of the pool waters and of the supply water obtained at the laboratory tap. One cubic centimeter of the water to be tested was inoculated into a fermentation tube. The results of these experiments showed that *B. coli* was only slightly more abundant in these swimming pools than in the city water supply.

Previous to this investigation disinfectants were regularly used twice a week in these tanks. It was found that at the different pools different strengths of disinfectant were being used and applied in different ways. It was desirable to secure the application of the same disinfectant to the three pools in more definite amounts as well as to make chemical tests on the water as the work proceeded. Mr. George W. Heise, chemist, Bureau of Science, coöperated and made all the chemical tests [19]

[19] *This Journal, Sec. A* (1916), 11, 105.

for this work and also supplied the data under "Turbidity" given in Table XIV.

In order to study the effect of various disinfectants on swimming pools, the chemical additions recorded in Table D were made. The Roman numerals refer to the tables of bacteriological data in which the results of the additions enumerated are recorded.

TABLE D.—*Outline of tests on Manila swimming pools.*

Week.	Disinfectant.			Results in Table—
	Kind.	Quantity (parts per million).	Added—	
1915.				
Aug. 30 to Sept. 4	Chloride of lime		Twice a week	I
Sept. 20 to 25	do	[a]0.5	Once a week	II
Sept. 27 to Oct. 2	do	[a]1.0	do	III
Oct. 4 to 9	do	[a]2.0	do	IV
Oct. 11 to 16	Copper sulphate	1.0	do	V
Oct. 18 to 22	do	2.0	do	VI
Oct. 25 to 30	None	0.0		VII
Nov. 1 to 8	Chloride of lime	[a]0.5	Daily	VIII
Nov. 8 to 13[b]	do	[a]0.5	do	IX
Nov. 15 to 20	Chloride of lime or antiformin.	[a]0.25	do	X
Dec. 6 to 11	Chloride of lime	[a]0.25	do	XI

[a] As available chlorine. [b] Without change of water.

TABLE I.—*Calcium hypochlorite added twice a week to water in swimming pools, week of August 30 to September 4, 1915.*

[Bacteria per cubic centimeter of water.]

	Aug. 30.	Aug. 31.	Sept. 1.	Sept. 2.	Sept. 3.	Sept. 4.
Pool I:						
Agar, 25° C	21,500	2,000	2,100	5,000	3,000	1,000
Litmus lactose agar, 37° C	57,000	2,500	3,800	700	100	4,300
Congo red agar, 37° C	50,000	1,400	5,600	600	100	2,100
Average	43,000	2,000	3,800	2,800	1,100	2,200
Pool II:						
Agar, 25° C	69,000	4,800	5,800	1,000	400	600
Litmus lactose agar, 37° C	110,000	4,700	2,200	600	300	3,500
Congo red agar, 37° C	100,000	3,300	4,100	700	100	4,100
Average	93,000	4,300	4,000	800	270	2,700
Pool III:						
Agar, 25° C	2,900	2,000	7,000	5,000	200	----
Litmus lactose agar, 37° C	3,000	2,700	8,000	500	900	----
Congo red agar, 37° C	5,000	2,100	3,200	800	300	----
Average	3,600	2,300	4,800	2,100	500	----

TABLE I.—*Calcium hypochlorite added twice a week to water in swimming pools, week of August 30 to September 4, 1915*—Continued.

[Bacteria per cubic centimeter of water—Continued.]

	Aug. 30.	Aug. 31.	Sept. 1.	Sept. 2.	Sept. 3.	Sept. 4.
Pool I:						
Bacillus coli test of 1 cc. water—						
Lactose bile	0	+	0	0	0	+
Litmus lactose agar		+				+
Coli-like colonies on original plates of—						
Litmus lactose agar	0	0	0	0	0	0
Congo red agar	0	+	0	0	0	+
Pool II:						
Bacillus coli test of 1 cc. water—						
Lactose bile	0	0	0	0	0	0
Litmus lactose agar						
Coli-like colonies on original plates of—						
Litmus lactose agar	0	0	0	0	0	0
Congo red agar	0	0	0	0	0	0
Pool III:						
Bacillus coli test of 1 cc. water—						
Lactose bile	+	+	+	+	+	
Litmus lactose agar	+	+	+	+	+	
Coli-like colonies on original plates of—						
Litmus lactose agar	0	0	0	0	0	
Congo red agar	0	+	0	0	0	

TABLE II.—*One-half part per million of available chlorine as calcium hypochlorite, added weekly to water in swimming pools, week of September 20 to 25, 1915.*

[Bacteria per cubic centimeter of water.]

	Sept. 20.	Sept. 21.	Sept. 22.	Sept. 23.	Sept. 24.	Sept. 25.
Pool I:						
Agar, 25° C.	20	100	10,000	80,000	40,000	100,000
Agar, 37° C.	50	120	10,500	60,000	60,000	170,000
Average	35	110	10,250	70,000	50,000	135,000
Pool II:						
Agar, 25° C.	3,500	400	12,000	10,000	10,000	110,000
Agar, 37° C.	4,000	600	13,000	16,000	20,000	150,000
Average	3,750	500	12,500	13,000	15,000	130,000
Pool III:						
Agar, 25° C.	40,000		2,400	100,000	100,000	1,900,000
Agar, 37° C.	50,000		3,000	100,000	120,000	2,200,000
Average	45,000		2,700	100,000	110,000	2,050,000

TABLE III.—*One part per million of available chlorine as calcium hypochlorite, added weekly to water in swimming pools, week of September 27 to October 2, 1915.*

[Bacteria per cubic centimeter of water.]

	Sept. 27.	Sept. 28.	Sept. 29.	Sept. 30.	Oct. 1.	Oct. 2.
Pool I:						
Agar, 25° C	900	30	18,000	42,000	120,000	200,000
Agar, 37° C	800	60	25,000	200,000	570,000	180,000
Average	850	45	21,500	121,000	345,000	190,000
Pool II:						
Agar, 25° C	52,000	110	17,000	14,000	22,000	90,000
Agar, 37° C	57,000	200	19,000	76,000	68,000	140,000
Average	54,500	155	18,000	45,000	45,000	115,000
Pool III:						
Agar, 25° C		5,000	190,000	64,000	500,000	170,000
Agar, 37° C		7,000	320,000	150,000	630,000	160,000
Average		6,000	255,000	107,000	565,000	165,000

TABLE IV.—*Two parts per million of available chlorine as calcium hypochlorite, added weekly to water in swimming pools, week of October 4 to 9, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 4.	Oct. 5.	Oct. 6.	Oct. 7.	Oct. 8.	Oct. 9.
Pool I:						
Agar, 25° C	4,000	70	2	11,000	1,300,000	150,000
Agar, 37° C	3,000	1,000	10	12,000	2,400,000	200,000
Average	3,500	550	6	11,500	1,850,000	175,000
Pool II:						
Agar, 25° C	1,000	100	22,000	20,000	40,000	200,000
Agar, 37° C	2,000	400	27,000	30,000	630,000	240,000
Average	1,500	250	24,500	25,000	335,000	220,000
Pool III:						
Agar, 25° C	6,000	70	4	55,000	1,200,000	6,000
Agar, 37° C	19,000	210	10	45,000	1,270,000	7,000
Average	12,500	140	7	50,000	1,235,000	6,500

TABLE V.—*One part per million copper sulphate, added weekly to water in swimming pools, week of October 11 to 16, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 11.	Oct. 12.	Oct. 13.	Oct. 14.	Oct. 15.	Oct. 16.
Pool I:						
Agar, 25° C	60	2,000	34,000	100,000	400,000	120,000
Agar, 37° C	30	1,700	57,000	360,000	500,000	210,000
Average	45	1,850	45,000	220,000	450,000	165,000
Pool II:						
Agar, 25° C	400	100	3,400	5,100,000	400,000	210,000
Agar, 37° C	220	80	17,000	5,700,000	800,000	300,000
Average	310	90	10,200	5,400,000	600,000	255,000
Pool III:						
Agar, 25° C	22,800	600	1,000	110,000	30,000	----------
Agar, 37° C	28,600	110,000	200,000	120,000	80,000	----------
Average	26,700	55,300	100,500	115,000	55,000	----------

TABLE VI.—*Two parts per million copper sulphate, added weekly to water in swimming pools, week of October 18 to 23, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 18.	Oct. 19.	Oct. 20.	Oct. 21.	Oct. 22.	Oct. 23.
Pool I:						
Agar, 25° C	500	28,000	170,000	1,800,000	5,100,000	17,000,000
Agar, 37° C	600	18,000	220,000	2,800,000	6,300,000	18,000,000
Average	550	23,000	195,000	2,300,000	5,700,000	17,500,000
Pool II:						
Agar, 25° C	900	20,000	570,000	2,300,000	5,400,000	----------
Agar, 37° C	12,000	19,000	280,000	3,900,000	6,900,000	----------
Average	10,500	19,500	425,000	3,100,000	6,150,000	----------
Pool III:						
Agar, 25° C	120,000	160,000	20,000	300	3,000	2,800,000
Agar, 37° C	130,000	150,000	50,000	9,000	4,000	4,800,000
Average	125,000	155,000	35,000	4,650	3,500	3,800,000

TABLE VII.—*No disinfectant added to water in swimming pools, week of October 25 to 30, 1915.*

[Bacteria per cubic centimeter of water.]

	Oct. 25.	Oct. 26.	Oct. 27.	Oct. 28.	Oct. 29.	Oct. 30.
Pool I:						
Agar, 25° C.	60	1,600	12,000	240,000	80,000	380,000
Agar, 37° C.	500	17,000	16,000	300,000	100,000	720,000
Average	280	9,300	14,000	270,000	90,000	550,000
Pool II:						
Agar, 25° C.	50	28,000	100,000	120,000	500,000	800,000
Agar, 37° C.	1,000	40,000	120,000	180,000	600,000	1,400,000
Average	525	34,000	110,000	150,000	550,000	1,100,000
Pool III:						
Agar, 25° C.			19,000	40,000	90,000	940,000
Agar, 37° C.			29,000	100,000	200,000	1,500,000
Average			24,000	70,000	145,000	1,220,000

TABLE VIII.—*One-half part per million of available chlorine as calcium hypochlorite, added daily to water in swimming pools, week of November 1 to 6, 1915.*

[Bacteria per cubic centimeter of water.]

	Nov. 1.	Nov. 2.	Nov. 3.	Nov. 4.	Nov. 5.	Nov. 6.
Pool I:						
Agar, 25° C.	100	30	60	0	80	0
Agar, 37° C.	200	100	80	40	300	6
Average	150	65	70	20	190	3
Pool III:						
Agar, 25° C.	30,000		40	170	100	
Agar, 37° C.	340,000		100	280	560	500
Average			70	225	330	

TABLE IX.—*One-half part per million of available chlorine as calcium hypochlorite, added daily to water in swimming pools, week of November 8 to 13, 1915. Second week in use.*

[Bacteria per cubic centimeter of water.]

	Nov. 8.	Nov. 9.	Nov. 10.	Nov. 11.	Nov. 12.	Nov. 13.
Pool I:						
Agar, 25° C.	20,000	2,700	60	30	200	10
Agar, 37° C.	23,000	9,600	400	170	10,000	300
Average	21,500	6,150	230	100	5,100	155
Pool III:						
Agar, 25° C.	68,000	400	40	2,300	30	100
Agar, 37° C.	60,000	1,000	800	5,700	1,600	600
Average	64,000	700	420	4,000	815	350

TABLE X.—*One-half part per million of available chlorine as calcium hypochlorite, added daily to pool III. One-seventh part per million of available chlorine as antiformin, added daily to pool I. Week of November 15 to 20, 1915.*

[Bacteria per cubic centimeter of water.]

	Nov. 15.	Nov. 16.	Nov. 17.	Nov. 18.	Nov. 19.	Nov. 20.
Pool I:						
Agar, 25° C	1,300	1,500	500	1,000	16,000	20,500
Agar, 37° C	2,400	19,000	10,000	4,000	34,000	42,000
Average	1,850	10,000	5,250	2,500	25,000	31,250
Pool III:						
Agar, 25° C	400	3,000	220	300	30	16
Agar, 37° C	800	5,100	7,100	2,000	80	20
Average	600	4,050	3,700	1,150	55	18

TABLE XI.—*One-fourth part per million of available chlorine as calcium hypochlorite, added daily to water in swimming pools, week of December 6 to 11, 1915.*

[Bacteria per cubic centimeter of water.]

	Dec. 6.	Dec. 7.	Dec. 8.	Dec. 9.	Dec. 10.	Dec. 11.
Pool I:						
Agar, 25° C	20	10	30	10	20	160
Agar, 37° C	160	30	210	100	380	390
Average	90	20	120	55	200	275
Pool III:						
Agar, 25° C	60	30	130	2,700	1,200	29,000
Agar, 37° C	240	600	2,900	28,500	13,000	44,000
Average	150	325	1,500	15,600	12,500	36,500

TABLE XII.—*Average bacterial counts of pools.*

[Bacteria per cubic centimeter of water.]

25° INCUBATION.

Table.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
II	1,760	250	8,130	63,300	50,000	703,000
III	26,450	1,713	75,000	40,000	214,000	153,300
IV	3,670	80	7,330	28,670	846,700	118,700
V	7,750	900	12,800	1,770,000	276,670	165,000
VI	700	24,000	370,000	2,050,000	5,250,000	17,000,000
VII	55	14,800	43,330	133,330	223,330	706,670
VIII	100	30	50	85	90	0
IX	44,000	1,550	50	1,165	115	55
X I	1,300	1,500	500	1,000	16,000	20,500
X III	400	3,000	220	300	30	16
XI	40	30	105	1,355	610	14,580
Average	7,838	4,350	47,046	371,745	625,231	1,716,529

TABLE XII.—*Average bacterial counts of pools*—Continued.

37° INCUBATION.

Table.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
II	4,025	360	5,500	58,600	66,600	846,600
III	28,900	2,420	121,300	142,000	422,600	160,000
IV	8,000	537	9,006	29,000	1,433,300	147,000
V	9,600	37,260	91,300	2,060,000	460,000	255,560
VI	6,300	18,500	250,000	3,350,000	6,600,000	18,000,000
VII	750	28,500	55,000	193,330	300,000	1,040,000
VIII	200	100	90	160	430	253
IX	41,500	5,300	600	2,935	5,800	450
X I	2,400	19,000	10,000	4,000	34,000	42,000
X II	800	5,100	7,100	2,000	80	20
XI	200	315	1,555	14,300	6,690	22,195
Average	9,334	10,672	50,131	441,434	848,136	1,864,916

TABLE XIII.—*Summary of tests for Bacillus coli in swimming-pool waters.*

Table.	Disinfectant used in pool.			*Bacillus coli*, times present during one week.							
	Kind.	Quantity (parts per million).	Frequency.	Pool I.		Pool II.		Pool III.		Average.	
				1 cc.	10 cc.	1 cc.	10 cc.	1 cc.	10 cc.	1 cc.	10 cc.
I	Calcium hypochlorite	------	Twice a week.	2	----	0	----	5	----	2.23	------
II	Chlorine from calcium hypochlorite.	0.5	Weekly	1	4	1	4	1	2	1	3.33
III	----do	1.0	----do	0	4	0	4	2	4	0.66	4
IV	----do	2.0	----do	0	4	0	3	0	4	0	3.66
V	Copper sulphate	1.0	----do	0	3	0	2	0	2	0	2.33
VI	----do	2.0	----do	0	2	0	0	0	0	0	0.66
VII	None	------	------	1	4	1	3	0	2	0.66	3
VIII	Chlorine from calcium hypochlorite.	0.5	Daily	0	1	----	----	0	3	0	2
IX	Chlorine from calcium hypochlorite (2d week).	0.5	----do	1	1	----	----	2	2	2.5	2.5
X	Chlorine from calcium hypochlorite.	0.5	----do	----	----	----	----	0	0	0	0
X	Chlorine from antiformin	½	----do	2	2	----	----	----	----	2	2
XI	Chlorine from calcium hypochlorite.	0.25	----do	0	3	----	----	1	3	0.5	3

DISCUSSION OF TABLES

A study of the preceding tables shows the relative restraining effects on *Bacillus coli* of the different disinfectants used. During week I calcium hypochlorite was used, in the same manner as used previous to these tests, but the strength of the disinfectant was low, and adequate purification was not effected. During week II, when, at the beginning of the week, 0.5 part of available chlorine by chemical analysis per million parts of water was added in the form of calcium hypochlorite, the *B. coli*

count was lowered. This was reduced still further during week III, when 1 part of chlorine per million was used, and during week IV, when 2 parts of chlorine were applied. The results of the determinations for *B. coli* are not shown in Tables III to XI, but are summarized in Table XIII. Weeks V and VI show the efficiency of copper sulphate in killing *B. coli*. Although the 1 cubic centimeter portions showed no difference, 10 cubic centimeter inoculations showed (Table XIII) that the average of 2.33 positive *B. coli* tests per week, obtained when 1 part of copper sulphate per million parts of water was used, was reduced to 0.66 times when 2 parts were applied. During week VII, when no disinfectant was used, the *B. coli* count again increased, but during the following week it was reduced by the daily application of 0.5 part of chlorine. Continuing the same application for another week (Table IX), the increase in the *B. coli* count, as well as that of other bacteria, showed that it was a disadvantage to use the water in a tank longer than one week. When fresh water was again used, the daily addition of antiformin, equivalent to one-seventh part of available chlorine per million parts of water (Table X), reduced the *B. coli* count to 2, while the daily addition of 0.5 part of chlorine (as chloride of lime) brought it down to zero. In an attempt further to reduce the amount of chloride of lime, 0.25 part of available chlorine was added daily during week XI. However, *B. coli* again made its appearance and the total bacterial counts gradually increased, showing that this amount of disinfectant was insufficient.

The total bacterial counts, with daily variations and the effect of some of the disinfectants, are shown in fig. 1, where the Roman numerals refer to the tables from which the averages are taken. The 37°C. counts are higher than the 25°C. counts, and in both cases the curve obtained when no disinfectant was added (VII) is highest. When 0.5 part of chlorine per million parts of water was used once a week, the bacteria were kept down somewhat, but at the end of the week they were nearly as numerous as in the former case. However, III (1 part of chlorine weekly) shows a considerable bacterial reduction at the end of the week. This reduction is more pronounced in the case of IV (2 parts of chlorine weekly). The most efficient disinfection was obtained when 0.5 part of chlorine (from chloride of lime) was added daily. This is represented by the dotted line VIII. In spite of the fact that curves based upon bacterial count often show surprisingly great jumps, which cannot be accounted for, these curves illustrate the conditions very

well and would probably show greater uniformity if a larger number of tests had been made or a greater number of pools could have been examined.

By comparing the bacterial counts of pools I and III in Tables VIII, IX, and XI, it is noticeable, especially in the last table, that the calcium hypochlorite seemed to have less disinfecting power in pool III than in pool I. This was true for both *B. coli* and total counts. In seeking a possible explanation, we find that it was not due to the difference in the number of bathers, as the daily average, during these weeks, of the people who used pool I was 19, whereas it was only 17 for pool III. The probable reason seems to be that the latter pool was open at the sides, admitting an abundance of light which caused a deterioration of the disinfectant. That light hastens the decomposition of the hypochlorite has already been mentioned.

During weeks VIII and IX the same water was used with the daily addition of 0.5 part of available chlorine from calcium hypochlorite per million parts of water. However, on the intervening Sunday no disinfectant was added. The effect was readily observable by the sudden increase of the bacterial count on the following day.

RELATION OF TURBIDITY AND BACTERIAL CONTENT

As the strength of a bacterial emulsion is often judged from its turbidity, it was thought that possibly some relation might exist between the turbidity of a swimming-pool water and its bacterial content. However, in this case, besides the physical suspension of bacteria, new chemical compounds are formed by the addition of disinfectants. Table XIV is presented to show how these factors compare.

TABLE XIV.—*Comparison of turbidity and bacterial content of water in swimming pools.*

[Average of 25° and 37° C. count.]

POOL I.

Age of water in days.	VII. No disinfectant.		V. One part per million copper sulphate.		VI. Two parts per million copper sulphate.		IV. Two parts per million chlorine from calcium hypochlorite.	
	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.
1	13.5	280	11.5	45	12.8	550	.....	3,500
2	8.5	9,300	10.5	1,850	13	23,000	13	550
3	.....	14,000	10.7	45,500	12	195,000	15	6
4	8.4	270,000	10.5	230,000	13.5	2,300,000	10	11,500
5	8.4	90,000	10.5	450,000	12.4	5,700,000	10.5	1,850,000
6	8.8	550,000	9.7	165,000	.....	17,500,000	13.5	175,000

TABLE XIV.—*Comparison of turbidity and bacterial content of water in swimming pools*—Continued.

POOL III.

Age of water in days.	VII. No disinfectant.		V. One part per million copper sulphate.		VI. Two parts per million copper sulphate.		IV. Two parts per million chlorine from calcium hypochlorite.	
	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.	Turbidity.	Bacteria per cc.
1	.....	.....	11.7	26,700	.....	125,000	16	12,500
2	.....	.....	11.3	55,200	20	150,000	10.1	140
3	15.5	24,000	11.3	100,500	12	35,000	10.1	7
4	14.5	70,000	9.7	115,000	.....	4,650	12.5	50,000
5	15	145,000	10.5	55,000	14	3,500	12.9	123,500
6	15.5	1,220,000	.....	.....	13	3,800,000	10.5	6,500

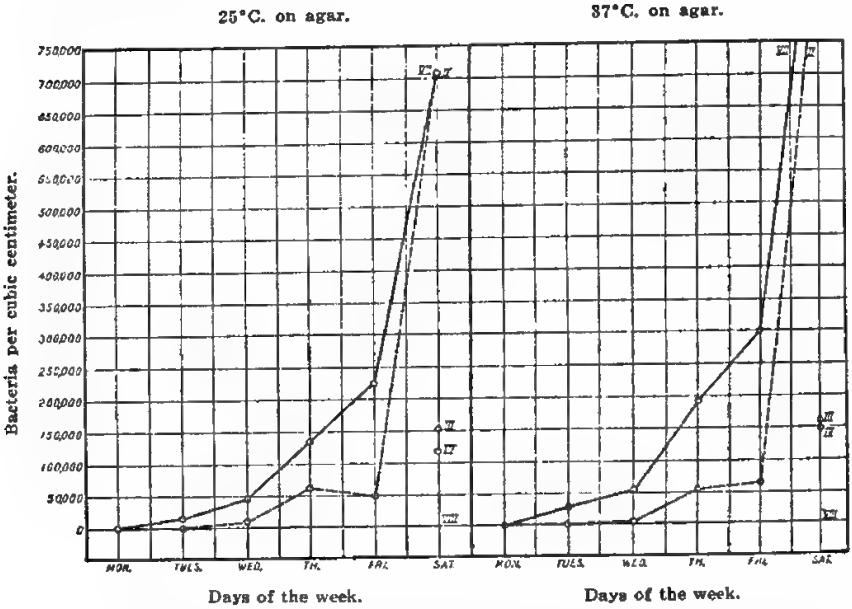

FIG. 1. Average daily counts of bacterial growth on agar at 25°C. and 37°C. The solid line was plotted from the data in Table VII; the dash line, from the data in Table II; and the dot line, from Table VIII. Only the respective end points are plotted for the curves resulting from the data in Tables III and IV.

As was anticipated, the addition of the disinfectants caused in pool I an increase in turbidity, further increased with larger amounts of disinfectant. Copper sulphate produced a greater turbidity than calcium hypochlorite. Pool III showed the same relations except that the recorded turbidity of the water to which no disinfectant was added was usually high. While in general an increased turbidity was associated with a decreased

bacterial content, no uniform relation between the two could be established.

SUMMARY AND CONCLUSIONS

Experiments have been made on three pools of 200 to 225 cubic meters' capacity, supplied with city water, which is a chlorinated river water with a temperature of about 28°C. The one hundred eighty-nine samples of 50 cubic centimeters of water were obtained about 1 meter below the surface and plated about forty minutes later on agar. After incubating at 25° and 37°C. for twenty-four hours, counts were made, and the positive presumptive tests for *B. coli* were further tested by plating.

Congo red lactose agar gave good results, but Endo medium seemed preferable for the confirmatory tests for *B. coli*. In the fermentation tubes lactose bile without peptone did not give as good results as lactose neutral red bouillon or lactose bouillon. One and 10 cubic centimeters of the water were inoculated, the latter into 30 cubic centimeters of the medium. *Bacillus coli* was found in the swimming pools to be usually not much more abundant than is accepted as permissible in drinking water. It was reduced most effectively by adding once a week 2 parts of copper sulphate per million parts of water. One week the daily addition of 0.5 part chlorine as calcium hypochlorite per million parts of water gave better results, while during another week the results were not as good as when the copper sulphate was used.

Copper sulphate produced a greater turbidity than calcium hypochlorite. An increase of disinfectant caused an increase in turbidity. Conversely, from the condition we find in a suspension of bacteria in water, due to the addition of a disinfectant, the number of bacteria often varied inversely as the turbidity, but not invariably. Consequently the determination of the turbidity would not be a practical test for determining the pollution in these pools. This can best be shown by regular tests for *B. coli* and the number of other bacteria present.

When a swimming pool is merely emptied and refilled, the bacterial count of the fresh water is much higher than when the emptied pool is thoroughly cleaned before refilling.

An increased amount of a given disinfectant invariably reduced the average numbers of *B. coli* and the total bacterial counts, as is graphically shown in fig. 1. This curve also shows the relation of the counts at 37°C. and those at 25°C., the former amounting to 116 per cent of the latter.

The general results of the experiments, as far as the bacterial count is concerned, may be summarized as follows:

1. When no disinfectant was added, there was a steady increase in the number of bacteria in the swimming pools. This increase was exceedingly high on the last day of the week the water was used.
2. When the disinfectants were used, the bacterial curve was lowered and often became more irregular.
   a. Copper sulphate applied at the beginning of the week permitted an increase throughout the week, but sometimes a slight reduction occurred.
   b. Calcium hypochlorite applied once a week showed good effects at first, but permitted too large an increase of bacteria toward the end of the week. A daily addition amounting to 0.25 part of available chlorine per million parts of water permitted a considerable increase of bacteria, while 0.5 part gave the best results.

When the comparative costs and disinfecting power are considered, antiformin and copper sulphate are not as valuable as calcium hypochlorite. The latter, when added daily in amounts equivalent to 0.5 part chlorine per million parts water, is recommended for the disinfection of these swimming pools, as it is the best and most economical means of keeping the number of intestinal and other bacteria within safe limits throughout the week in which the same water is used.

ILLUSTRATION

TEXT FIGURE

FIG. 1. Chart, showing average daily counts of bacterial growth on agar at 25°C. and 37°C. The solid line was plotted from the data in Table VII; the dash line, from the data in Table II; and the dot line, from Table VIII. Only the respective end points are plotted for the curves resulting from the data in Tables III and IV.

WASSERMANN REACTION WITH GLYCERINATED HUMAN SERUM [1]

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In a previous report [2] I showed that glycerin is a suitable preservative for human serum intended for the Wassermann reaction. It prevents bacterial growth and does not materially influence the test. The sera previously reported on were studied for a short time only and were tested at weekly intervals; this report deals with sera tested monthly for a period of three months.

Method.—The Wassermann method with human hæmolytic system was used exclusively in this investigation.

Complement.—As complement serum the pooled sera of three guinea pigs were used in quantities of 0.1, 0.05, and 0.025 cubic centimeter.

Antigen.—The antigen was alcoholic extract of human-heart muscle, and was used in quantities of about one fourth of the anticomplementary dose for 1.25 unit of hæmolytic amboceptor with 0.05 cubic centimeter of complement serum. This may be done provided it is not anticomplementary in the antigen control, or an allowance is made when the results are read. If one fourth of the anticomplementary dose is anticomplementary in the antigen control, I prefer to use the largest quantity of antigen that does not show any anticomplementary property in the antigen control. The control should never be omitted. Throughout this investigation 1 cubic centimeter of alcoholic extract was diluted with 29 cubic centimeters of physiologic salt solution, and each tube received 0.5 cubic centimeter of the diluted antigen.

Hæmolytic amboceptor.—The serum of rabbits that had been immunized against washed human-blood corpuscles was used in doses of from 1 to 2 units per test tube. The term unit was

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[2] *This Journal, Sec. B* (1916), 11, 1.

applied to the smallest quantity of hæmolytic serum which with 0.05 cubic centimeter of complement dissolved the test dose of sensitized corpuscles in one hour. The mixture of hæmolytic amboceptor and corpuscles was allowed to stand at room temperature for thirty minutes before the complement was added. After the complement had been added, the tubes were placed in the incubator at 37°C. for one hour.

Corpuscles.—Human corpuscles from nonsyphilitic persons were well washed and were used in doses of 0.5 cubic centimeter of a 4 per cent suspension in physiologic salt solution. The corpuscles were sensitized for thirty minutes before they were added to the serum-complement-antigen mixture.

Technique.—The technique used in this investigation was identical with that described in the previous report. Before testing, the human sera were heated to about 55.5°C. (the temperature varied from 55.2°C. to 55.7°C.) for thirty minutes. Six-tenths cubic centimeter of the inactivated serum or 1.2 cubic centimeters of equal parts of human serum and chemically pure glycerin were diluted to 3 cubic centimeters with physiologic salt solution. Six test tubes, 1, 2, and 3 as antigen tubes and 1′, 2′, and 3′ as control tubes, were used in each test. Each tube received 0.5 cubic centimeter of diluted serum. I preferred to use the constant quantity of serum in order to have the anticomplementary property uniform in all tubes. Each of the first pair of tubes, tubes 1 and 1′, received 0.5 cubic centimeter of 1:5 dilution of complement serum; each tube of the second pair, tubes 2 and 2′, received 0.5 cubic centimeter of 1:10 dilution of complement serum; and each of the third pair of tubes, tubes 3 and 3′, received 0.5 cubic centimeter of 1:20 dilution of complement serum. Each of the antigen tubes, tubes 1, 2, and 3, received 0.5 cubic centimeter of 1:30 dilution of alcoholic extract (antigen); and the control tubes, tubes 1′, 2′, and 3′, received 0.5 cubic centimeter of physiologic salt solution each. These mixtures were placed in the incubator at 37°C. for one hour. After having been in the incubator one hour, each tube received 1 cubic centimeter of sensitized corpuscles, representing 0.5 cubic centimeter of 4 per cent suspension of washed corpuscles and 1 unit of amboceptor diluted to 0.5 cubic centimeter with physiologic salt solution. After shaking, the tubes were placed in the incubator at 37°C. for one hour; during this hour and during the thirty minutes while the corpuscles were being sensitized, the mixtures were repeatedly shaken to prevent the

corpuscles from settling to the bottom of the container. After having been in the incubator for one hour, the tubes were allowed to stand at room temperature for two hours, after which the first reading was taken. After the first reading the tubes were put into the refrigerator, and the final results were read on the following morning.

Antigen control.—Six test tubes were used as in conducting the test, but the human serum was omitted, and the volume was made up with physiologic salt solution. If there was no anticomplementary action in the antigen control, the dose of antigen was considered suitable. If there was anticomplementary action, the dose of antigen was decreased until there was no anticomplementary action.

TEST 1

Specimens 4424, 4425, 4426, 4427, and 4428 were secured November 23, 1915. The sera were drawn off the clots the next day, and each serum was divided into two portions, A and B. Unglycerinated, portion A was tested November 24. Portion B, unheated, was mixed with an equal volume of sterilized, chemically pure glycerin and was kept at room temperature in a cork-stoppered test tube.

Specimens 4429, 4430, 4432, and 4433 were secured November 24, 1915. The sera were drawn off the clots the next day, and each serum was divided into two portions, A and B. Portion A, unglycerinated, was tested November 25. Unheated, portion B was mixed with an equal volume of sterilized, chemically pure glycerin and was kept at room temperature in a cork-stoppered test tube.

Specimen 4434 was secured November 26, 1915. The serum was drawn off the clot the next day and was divided into two portions, A and B. Unglycerinated, portion A was tested November 27. Unheated, portion B was mixed with an equal volume of sterilized, chemically pure glycerin and was kept at room temperature in a cork-stoppered test tube.

Portion B of each of the above sera was tested December 26, 1915, January 30, 1916, and February 22, 1916. Immediately before testing, the glycerinated serum (1.2 cubic centimeters) necessary for the test was heated to about 55.5°C. for thirty minutes.

All sera were tested bacteriologically.

TABLE I.—*Wassermann reaction with glycerinated human sera, heated immediately before testing.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.									
		A	Nov. 24	Nov. 24	1.0	1	0	0	0	+	±	0	Strongly positive.
						2	tr	0	0	+	+	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	0	0	0	+	±	0	Do.
4424	Nov. 23					2	0	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	0	0	+	+	+	Do.
						2	+	0	0	+	+	+	Do.
			1915.	1915.									
		A	Nov. 24	Nov. 24	1.0	1	+	tr	0	+	+	0	Moderately positive.
						2	+	±	0	+	+	tr	Do.
		B	Dec. 26	Dec. 26	1.25	1	+	0	0	+	±	0	Do.
4425	...do.....					2	+	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	+	0	0	+	±	0	Do.
						2	+	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	+	0	+	+	±	Do.
						2	+	+	0	+	+	±	Do.
			1915.	1915.									
		A	Nov. 24	Nov. 24	1.0	1	tr	0	0	+	±	0	Strongly positive.
						2	tr	0	0	+	±	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	0	0	0	+	tr	0	Do.
4426	...do.....					2	0	0	0	+	tr	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	±	0	0	+	±	0	Do.
						2	±	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	tr	0	+	+	±	Do.
						2	+	tr	0	+	+	±	Do.
			1915.	1915.									
		A	Nov. 24	Nov. 24	1.0	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	0	0	0	+	±	0	Do.
4427	...do.....					2	0	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.
			1915.	1915.									
		A	Nov. 24	Nov. 24	1.0	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	0	0	0	+	±	0	Do.
4428	...do.....					2	0	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	tr	0	0	+	+	0	Do.
						2	tr	0	0	+	+	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.

TABLE I.—*Wassermann reaction with glycerinated human sera, heated immediately before testing*—Continued.

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
			1915.	1915.									
		A	Nov. 24	Nov. 24	1. 0	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	0	0	0	+	±	0	Do.
4429	Nov. 23					2	0	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	0	0	+	+	tr	Do.
						2	+	0	0	+	+	tr	Do.
			1915.	1915.									
		A	Nov. 25	Nov. 25	1.0	1	+	±	0	+	+	0	Weakly positive.
						2	+	±	0	+	+	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	+	tr	0	+	±	0	Do.
4430	Nov. 24					2	+	tr	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	+	tr	0	+	±	0	Moderately positive.
						2	+	tr	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	+	tr	+	+	±	Do.
						2	+	+	tr	+	+	±	Do.
			1915.	1915.									
		A	Nov. 25	Nov. 25	1.0	1	0	0	0	+	±	0	Strongly positive.
						2	0	0	0	+	±	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	0	0	0	+	±	0	Do.
4432	...do					2	0	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	0	0	+	+	tr	Do.
						2	+	0	0	+	+	tr	Do.
			1915.	1915.									
		A	Nov. 25	Nov. 25	1.0	1	+	±	0	+	+	0	Weakly positive.
						2	+	±	0	+	+	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	+	0	0	+	tr	0	Moderately positive.
4433	...do					2	±	0	0	+	tr	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	+	0	0	+	±	0	Do.
						2	+	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	+	0	+	+	tr	Weakly positive.
						2	+	+	0	+	+	tr	Do.
			1915.	1915.									
		A	Nov. 25	Nov. 25	1.0	1	tr	0	0	+	±	0	Strongly positive.
						2	tr	0	0	+	±	0	Do.
		B	Dec. 26	Dec. 26	1.25	1	tr	0	0	+	±	0	Do.
4434	...do					2	tr	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 30	Jan. 30	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
		B	Feb. 22	Feb. 22	1.5	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.

Explanation: +, complete hæmolysis; ±, hæmolysis between 50 per cent and 100 per cent; tr (trace), hæmolysis less than 50 per cent; 0, no hæmolysis.

Table I shows that with these ten sera, namely, Nos. 4424, 4425, 4426, 4427, 4428, 4429, 4430, 4432, 4433, and 4434, glycerin did not influence the Wassermann reaction. Sera 4430 and 4433 gave weakly positive results at the first test and moderately positive results at subsequent tests. With the other sera subsequent tests gave results practically identical with the results of the first tests. After having been kept at room temperature for three months, these sera were bacteriologically sterile, and after having been heated to about 55.5°C. for thirty minutes, they were but slightly anticomplementary. There was little or no difference between the two readings.

TEST 2

Specimens 4435, 4436, 4437, 4438, and 4439 were secured November 26, 1915. The next day the sera were drawn off the clots, and each serum was divided into two portions, A and B. Without having been mixed with glycerin, portion A of each serum was tested November 27. Portion B of each serum was heated to about 55.5°C. for thirty minutes November 27, was mixed with an equal volume of sterilized, chemically pure glycerin, and was kept at room temperature in a cork-stoppered test tube to be tested later.

Specimens 4440, 4441, 4442, 4443, and 4444 were secured November 27. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested November 28. On the same date portion B of each serum was heated to about 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, and was kept at room temperature in a cork-stoppered test tube to be tested later.

Without having been reheated, portion B of each serum was tested December 27, 1915, January 30, 1916, and February 27, 1916.

February 27, 1916, each serum was tested bacteriologically.

Table II shows that with the ten sera used in test 2 the glycerin did not noticeably influence the Wassermann reaction. After having been kept at room temperature for three months, the results obtained with the test were practically identical with the results obtained before the sera had been mixed with glycerin. The sera had not become anticomplementary and were free from bacterial growth. There was little or no difference between the reading taken three hours after the corpuscles had been added and that taken eighteen hours after the corpuscles had been added.

TABLE II.—*Wassermann reaction with human sera, heated before having been mixed with the glycerin.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.									
		A	Nov. 27	Nov. 27	1.0	1	tr	0	0	+	+	0	Strongly positive.
						2	tr	0	0	+	+	0	Do.
		B	...do.....	Dec. 27	1.25	1	±	0	0	+	+	tr	Do.
						2	±	0	0	+	+	tr	Do.
4435	Nov. 26			1916.									
		B	...do.....	Jan. 30	1.25	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.
		B	...do.....	Feb. 27	1.25	1	tr	0	0	+	tr	0	Do.
						2	tr	0	0	+	tr	0	Do.
				1915.									
		A	...do.....	Nov. 27	1.0	1	+	tr	0	+	±	0	Weakly positive.
						2	+	±	0	+	+	0	Do.
		B	...do.....	Dec. 27	1.25	1	+	±	0	+	+	tr	Moderately positive.
						2	+	±	0	+	+	tr	Do.
4436	...do.....			1916.									
		B	...do.....	Jan. 30	1.25	1	+	+	0	+	+	tr	Weakly positive.
						2	+	+	0	+	+	tr	Do.
		B	...do.....	Feb. 27	1.25	1	±	0	0	+	tr	0	Moderately positive.
						2	±	0	0	+	tr	0	Do.
				1915.									
		A	...do.....	Nov. 27	1.0	1	0	0	0	+	±	0	Strongly positive.
						2	0	0	0	+	±	0	Do.
		B	...do.....	Dec. 27	1.25	1	tr	0	0	+	+	tr	Do.
						2	tr	0	0	+	+	tr	Do.
4437	...do.....			1916.									
		B	...do.....	Jan. 30	1.25	1	±	0	0	+	+	±	Do.
						2	±	0	0	+	+	±	Do.
		B	...do.....	Feb. 27	1.25	1	tr	0	0	+	±	0	Do.
						2	tr	0	0	+	±	0	Do.
				1915.									
		A	...do.....	Nov. 27	1.0	1	+	0	0	+	+	tr	Do.
						2	+	0	0	+	+	tr	Do.
		B	...do.....	Dec. 27	1.25	1	±	0	0	+	+	tr	Do.
						2	±	0	0	+	+	tr	Do.
4438	...do.....			1916.									
		B	...do.....	Jan. 30	1.25	1	+	0	0	+	+	±	Do.
						2	+	0	0	+	+	±	Do.
		B	...do.....	Feb. 27	1.25	1	±	0	0	+	+	0	Do.
						2	±	0	0	+	+	0	Do.
				1915.									
		A	...do.....	Nov. 27	1.0	1	+	±	0	+	+	0	Weakly positive.
						2	+	±	0	+	+	0	Do.
		B	...do.....	Dec. 27	1.25	1	+	+	0	+	+	±	Moderately positive.
						2	+	+	0	+	+	±	Do.
4439	...do.....			1916.									
		B	...do.....	Jan. 30	1.25	1	+	+	0	+	+	±	Do.
						2	+	+	0	+	+	±	Do.
		B	...do.....	...do.....	1.25	1	+	tr	0	+	+	0	Do.
						2	+	tr	0	+	+	0	Do.

TABLE II.—*Wassermann reaction with human sera, heated before having been mixed with the glycerin*—Continued.

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
			1915.	1915.		1	tr	0	0	+	+	tr	Strongly positive.
		A	Nov. 28	Nov. 28	1.0	2	tr	0	0	+	+	tr	Do.
						1	0	0	0	+	+	0	Do.
		B	...do.....	Dec. 27	1.25	2	0	0	0	+	+	0	Do.
4440	Nov. 27			1916.		1	0	0	0	+	+	tr	Do.
		B	...do.....	Jan. 30	1.25	2	0	0	0	+	+	tr	Do.
						1	0	0	0	+	+	0	Do.
		B	...do.....	Feb. 27	1.25	2	0	0	0	+	+	0	Do.
				1915.		1	0	0	0	+	+	tr	Do.
		A	...do.....	Nov. 28	1.0	2	0	0	0	+	+	tr	Do.
						1	0	0	0	+	±	0	Do.
		B	...do.....	Dec. 27	1.25	2	0	0	0	+	±	0	Do.
4441	...do.....			1916.		1	0	0	0	+	+	0	Do.
		B	...do.....	Jan. 30	1.25	2	0	0	0	+	+	0	Do.
						1	0	0	0	+	+	0	Do.
		B	...do.....	Feb. 27	1.25	2	0	0	0	+	+	0	Do.
				1915.		1	+	0	0	+	+	tr	Do.
		A	...do.....	Nov. 28	1.0	2	+	0	0	+	+	tr	Do.
						1	0	0	0	+	+	0	Do.
		B	...do.....	Dec. 27	1.25	2	0	0	0	+	+	0	Do.
4442	...do.....			1916.		1	0	0	0	+	+	0	Do.
		B	...do.....	Jan. 30	1.25	2	0	0	0	+	+	0	Do.
						1	0	0	0	+	+	tr	Do.
		B	...do.....	Feb. 27	1.25	2	0	0	0	+	+	tr	Do.
				1915.		1	tr	0	0	+	+	0	Do.
		A	...do.....	Nov. 28	1.0	2	tr	0	0	+	+	0	Do.
						1	0	0	0	+	+	0	Do.
		B	...do.....	Dec. 27	1.25	2	0	0	0	+	+	0	Do.
4443	...do.....			1916.		1	0	0	0	+	+	tr	Do.
		B	...do.....	Jan. 30	1.25	2	0	0	0	+	+	tr	Do.
						1	tr	0	0	+	+	tr	Do.
		B	...do.....	Feb. 27	1.25	2	tr	0	0	+	+	tr	Do.
				1915.		1	+	tr	0	+	±	0	Weakly positive.
		A	...do.....	Nov. 28	1.0	2	+	tr	0	+	±	0	Do.
						1	+	tr	0	+	±	0	Do.
		B	...do.....	Dec. 27	1.25	2	+	tr	0	+	±	0	Do.
4444	...do.....			1916.		1	+	+	0	+	+	tr	Do.
		B	...do.....	Jan. 30	1.25	2	+	+	0	+	+	tr	Do.
						1	+	+	0	+	+	tr	Do.
		B	...do.....	Feb. 27	1.25	2	+	+	0	+	+	tr	Do.

TEST 3

November 30, 1915, specimens 4446, 4447, 4448, 4449, and 4450 were secured. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested December 1. Portion B was heated to 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, was placed in the cold storage at about 7°C. in a cork-stoppered test tube, and was tested at intervals of about a month. About three months after it was mixed with glycerin it was examined bacteriologically.

Table III shows that with sera 4446, 4447, 4448, 4449, and 4450 glycerin did not influence the Wassermann reaction during the period of three months. The results obtained at the end of one month, at the end of two months, and at the end of three months were practically identical with the results obtained before glycerin had been added to the sera. The sera having been heated on December 1, 1915, before they had been mixed with glycerin, did not become anticomplementary in three months, and all remained free from bacterial growth.

TEST 4

Specimens 4452, 4453, 4454, 4455, and 4456 were secured December 1, 1915. The next day the sera were drawn off the clots. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested December 2. Portion B of each serum was mixed with an equal volume of sterilized, chemically pure glycerin, was placed in the cold storage at a temperature of about 7°C., and was tested at intervals of about a month. The necessary quantity of serum was heated immediately before the test.

On March 4, 1916, a bacteriologic test was made of each serum.

TABLE III.—*Wassermann reaction with glycerinated human sera, heated before having been mixed with glycerin; kept in cold storage.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.									
		A	Dec. 1	Dec. 1	1.0	1	+	0	0	+	+	tr	Strongly positive.
						2	+	0	0	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 2	1.25	1	tr	0	0	+	+	0	Do.
4446	Nov. 30					2	tr	0	0	+	+	0	Do.
		B	...do.....	Feb. 6	1.5	1	+	0	0	+	+	+	Do.
						2	+	0	0	+	+	+	Do.
		B	...do.....	Mar. 4	1.25	1	tr	0	0	+	±	0	Do.
						2	tr	0	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 1	1.0	1	0	0	0	+	+	0	Do.
						2	0	0	0	+	+	0	Do.
				1916.									
		B	...do.....	Jan. 2	1.25	1	0	0	0	+	+	0	Do.
4447	...do.....					2	0	0	0	+	+	0	Do.
		B	...do.....	Feb. 6	1.5	1	0	0	0	+	+	+	Do.
						2	0	0	0	+	+	+	Do.
		B	...do.....	Mar. 4	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 1	1.0	1	+	tr	0	+	+	tr	Do.
						2	+	tr	0	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 2	1.25	1	0	0	0	+	±	0	Do.
4448	...do.....					2	0	0	0	+	±	0	Do.
		B	...do.....	Feb. 6	1.5	1	+	0	0	+	+	+	Do.
						2	+	0	0	+	+	+	Do.
		B	...do.....	Mar. 4	1.25	1	tr	0	0	+	±	0	Do.
						2	tr	0	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 1	1.0	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 2	1.25	1	0	0	0	+	±	0	Do.
4449	...do.....					2	0	0	0	+	±	0	Do.
		B	...do.....	Feb. 6	1.5	1	0	0	0	+	+	+	Do.
						2	0	0	0	+	+	+	Do.
		B	...do.....	Mar. 4	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 1	1.0	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 2	1.25	1	0	0	0	+	±	0	Do.
4450	...do.....					2	0	0	0	+	±	0	Do.
		B	...do.....	Feb. 6	1.5	1	0	0	0	+	+	+	Do.
						2	0	0	0	+	+	+	Do.
		B	...do.....	Mar. 4	1.25	1	0	0	0	+	±	0	Do.
						2	0	0	0	+	±	0	Do.

TABLE IV.—*Wassermann reaction with glycerinated human sera kept in the cold storage, heated immediately before testing.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.									
		A	Dec. 2	Dec. 2	1.0	1	tr	0	0	+	+	tr	Strongly positive.
						2	tr	0	0	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 2	Jan. 2	1.25	1	0	0	0	+	0	0	Do.
4452	Dec. 1					2	0	0	0	+	0	0	Do.
		B	Feb. 6	Feb. 6	1.5	1	0	0	0	+	0	0	Do.
						2	0	0	0	+	0	0	Do.
		B	Mar. 4	Mar. 4	2.0	1	0	0	0	+	0	0	Do.
						2	0	0	0	+	0	0	Do.
			1915.	1915.									
		A	Dec. 2	Dec. 2	1.0	1	+	0	0	+	±	0	Moderately positive.
						2	+	0	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 2	Jan. 2	1.25	1	0	0	0	±	0	0	Do.
4453	...do					2	0	0	0	±	0	0	Do.
		B	Feb. 6	Feb. 6	1.5	1	0	0	0	+	0	0	Strongly positive.
						2	0	0	0	+	0	0	Do.
		B	Mar. 4	Mar. 4	2.0	1	0	0	0	+	0	0	Do.
						2	0	0	0	+	0	0	Do.
			1915.	1915.									
		A	Dec. 2	Dec. 2	1.0	1	tr	0	0	+	+	tr	Do.
						2	tr	0	0	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 2	Jan. 2	1.25	1	0	0	0	±	0	0	Positive.
4454	...do					2	0	0	0	±	0	0	Do.
		B	Feb. 6	Feb. 6	1.5	1	0	0	0	+	0	0	Strongly positive.
						2	0	0	0	+	0	0	Do.
		B	Mar. 4	Mar. 4	2.0	1	0	0	0	+	0	0	Do.
						2	---	---	---	---	---	---	Do.
			1915.	1915.									
		A	Dec. 2	Dec. 2	1.0	1	+	0	0	+	+	tr	Do.
						2	+	0	0	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 2	Jan. 2	1.25	1	0	0	0	±	0	0	Positive.
4455	...do					2	0	0	0	±	0	0	Do.
		B	Feb. 6	Feb. 6	1.5	1	0	0	0	+	0	0	Strongly positive.
						2	0	0	0	+	0	0	Do.
		B	Mar. 4	Mar. 4	2.0	1	0	0	0	+	0	0	Do.
						2	0	0	0	+	0	0	Do.
			1915.	1915.									
		A	Dec. 2	Dec. 2	1.0	1	0	0	0	+	+	tr	Do.
						2	0	0	0	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 2	Jan. 2	1.25	1	0	0	0	+	0	0	Do.
4456	...do					2	0	0	0	+	0	0	Do.
		B	Feb. 6	Feb. 6	1.5	1	0	0	0	+	0	0	Do.
						2	0	0	0	+	0	0	Do.
		B	Mar. 4	Mar. 4	2.0	1	0	0	0	+	0	0	Do.
						2	0	0	0	+	0	0	Do.

Table IV shows that with sera 4452, 4453, 4454, 4455, and 4456 the glycerin did not noticeably influence the Wassermann reaction. All of these sera became strongly anticomplementary, far more so than did the sera that were kept at room temperature. All sera remained free from bacterial growth.

TEST 5

Specimens 4535, 4536, 4537, 4538, and 4539 were secured December 27, 1915. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested on December 28. Portion B of each serum was mixed with an equal volume of sterilized, chemically pure glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of a month. The necessary quantity of serum was heated to 55.5° C. immediately before testing. A bacteriologic test was made of each serum on March 18, 1916.

Table V shows that with sera 4535, 4536, 4537, 4538, and 4539 the glycerin did not noticeably influence the Wassermann reaction. Although the sera had not been heated, they did not become very strongly anticomplementary in about three months. The eratic results obtained on February 13, 1916, are not easily explained. All sera remained clear and free from bacterial growth.

TEST 6

Specimens 4540, 4541, 4542, 4543, and 4544 were secured on December 27, 1915. December 28 the sera were drawn off the clots. Each serum was divided into two portions, A and B. Unglycerinated, portion A was tested December 28. Portion B was heated to about 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of about a month. On March 18, 1916, each serum was tested bacteriologically.

TABLE V.—*Wassermann reaction with glycerinated human sera, kept at room temperature, heated immediately before testing.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.		1	+	+	tr	+	+	tr	Negative.
		A	Dec. 28	Dec. 28	1.0	2	+	+	tr	+	+	tr	Do.
			1916.	1916.		1	+	tr	0	+	tr	0	Do.
		B	Jan. 23	Jan. 23	1.25	2	+	tr	0	+	tr	0	Do.
4535	Dec. 27	B	Feb. 13	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	Mar. 18	Mar. 18	1.5	1	+	±	0	+	±	0	Negative.
						2	+	±	0	+	±	0	Do.
			1915.	1915.		1	+	+	tr	+	+	tr	Do.
		A	Dec. 28	Dec. 28	1.0	2	+	+	tr	+	+	tr	Do.
			1916.	1916.		1	+	tr	0	+	tr	0	Do.
		B	Jan. 23	Jan. 23	1.25	2	+	tr	0	+	tr	0	Do.
4536	...do	B	Feb. 13	Feb. 13	1.5	1	+	0	0	+	0	0	Do.
						2	+	0	0	+	0	0	Do.
		B	Mar. 18	Mar. 18	1.5	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
			1915.	1915.		1	+	+	0	+	+	0	Do.
		A	Dec. 28	Dec. 28	1.0	2	+	+	0	+	+	0	Do.
			1916.	1916.		1	+	tr	0	+	tr	0	Do.
		B	Jan. 23	Jan. 23	1.25	2	+	tr	0	+	tr	0	Do.
4537	...do	B	Feb. 13	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	Mar. 18	Mar. 18	1.5	1	+	+	tr	+	+	tr	Negative.
						2	+	+	tr	+	+	tr	Do.
			1915.	1915.		1	+	+	0	+	+	0	Do.
		A	Dec. 28	Dec. 28	1.0	2	+	+	0	+	+	0	Do.
			1916.	1916.		1	+	tr	0	+	tr	0	Do.
		B	Jan. 23	Jan. 23	1.25	2	+	tr	0	+	tr	0	Do.
4538	...do	B	Feb. 13	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	Mar. 18	Mar. 18	1.5	1	+	+	tr	+	+	tr	Negative.
						2	+	+	tr	+	+	tr	Do.
			1915.	1915.		1	+	+	0	+	+	0	Do.
		A	Dec. 28	Dec. 28	1.0	2	+	+	0	+	+	0	Do.
			1916.	1916.		1	+	0	0	+	0	0	Do.
		B	Jan. 23	Jan. 23	1.25	2	+	0	0	+	0	0	Do.
4539	...do	B	Feb. 13	Feb. 13	1.5	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	Mar. 18	Mar. 18	1.5	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.

TABLE VI.—*Wassermann reaction with glycerinated human sera, heated before having been mixed with the glycerin, kept at room temperature.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.									
		A	Dec. 28	Dec. 28	1.0	1	+	+	tr	+	+	tr	Negative.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4540	Dec. 27					2	+	tr	0	+	tr	0	Do.
		B	...do.....	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	...do.....	Mar. 18	1.25	1	+	tr	0	+	tr	0	Negative.
						2	+	tr	0	+	tr	0	Do.
				1915.									
		A	...do.....	Dec. 28	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4541	...do.....					2	+	tr	0	+	tr	0	Do.
		B	...do.....	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	...do.....	Mar. 18	1.25	1	+	tr	0	+	tr	0	Negative.
						2	+	tr	0	+	tr	0	Do.
				1915.									
		A	...do.....	Dec. 28	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4542	...do.....					2	+	tr	0	+	tr	0	Do.
		B	...do.....	Feb. 13	1.5	1	+	+	±	+	+	±	Do.
						2	+	+	±	+	+	±	Do.
		B	...do.....	Mar. 18	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 28	1.0	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4543	...do.....					2	+	tr	0	+	tr	0	Do.
		B	...do.....	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	...do.....	Mar. 18	1.25	1	+	±	0	+	±	0	Negative.
						2	+	±	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 28	1.0	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4544	...do.....					2	+	tr	0	+	tr	0	Do.
		B	...do.....	Feb. 13	1.5	1	+	+	+	+	+	+	None.
						2	+	+	+	+	+	+	Do.
		B	...do.....	Mar. 18	1.25	1	+	±	0	+	±	0	Negative.
						2	+	±	0	+	±	0	Do.

Table VI shows the results obtained with sera 4540, 4541, 4542, 4543, and 4544. The glycerin did not noticeably influence the Wassermann reaction. The sera did not become anticomplementary. They remained clear and free from bacterial growth.

TEST 7

On December 29, 1915, specimens 4546, 4547, 4548, 4549, and 4550 were secured. The sera were drawn off the clots December 30. Each serum was divided into two portions, A and B. Unglycerinated, portion A of each serum was tested December 30. Unheated, portion B of each serum was mixed with an equal volume of sterilized, chemically pure glycerin, was kept in a cork-stoppered test tube in the cold storage at a temperature of about 7°C., and was tested at intervals of about a month. The necessary quantity of serum was heated to 55.5°C. for thirty minutes immediately before testing.

Table VII shows that with sera 4546, 4547, 4548, 4549, and 4550 the glycerin did not influence the Wassermann reaction. The sera did not become very strongly anticomplementary in about three months, and all sera remained clear and free from bacterial growth. There was practically no difference between the first and second readings.

TEST 8

Specimens 4551, 4552, 4553, 4554, and 4555 were secured December 29, 1915. The sera were drawn off the clots the next day. Each serum was divided into two portions, A and B. Unglycerinated, portion A of each serum was tested December 30. Portion B of each serum was heated to about 55.5°C. for thirty minutes, was mixed with an equal volume of sterilized, chemically pure glycerin, was kept in a cork-stoppered test tube in the cold storage at a temperature of about 7°C., and, without reheating, was tested at intervals of about a month. On March 25, 1916, each serum was tested for bacterial growth.

TABLE VII.—*Wassermann reaction with glycerinated human sera, kept in the cold storage, heated immediately before testing.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.		1	+	+	tr	+	+	tr	Negative.
		A	Dec. 30	Dec. 30	1.0	2	+	+	tr	+	+	tr	Do.
			1916.	1916.		1	+	±	0	+	±	0	Do.
		B	Jan. 23	Jan. 23	1.25	2	+	±	0	+	±	0	Do.
4546	Dec. 29					1	+	tr	0	+	tr	0	Do.
		B	Feb. 20	Feb. 20	1.25	2	+	tr	0	+	tr	0	Do.
		B	Mar. 25	Mar. 25	1.5	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
			1915.	1915.									
		A	Dec. 30	Dec. 30	1.0	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
			1916.	1916.									
		B	Jan. 23	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4547	...do					2	+	tr	0	+	tr	0	Do.
		B	Feb. 20	Feb. 20	1.25	1	+	0	0	+	0	0	Do.
						2	+	0	0	+	0	0	Do.
		B	Mar. 25	Mar. 25	1.5	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
			1915.	1915.									
		A	Dec. 30	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 23	Jan. 23	1.25	1	+	0	0	+	0	0	Do.
4548	...do					2	+	0	0	+	0	0	Do.
		B	Feb. 20	Feb. 20	1.25	1	+	0	0	+	0	0	Do.
						2	+	0	0	+	0	0	Do.
		B	Mar. 25	Mar. 25	1.5	1	+	0	0	+	0	0	Do.
						2	+	0	0	+	0	0	Do.
			1915.	1915.									
		A	Dec. 30	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 23	Jan. 23	1.25	1	+	tr	0	+	tr	0	Do.
4549	...do					2	+	tr	0	+	tr	0	Do.
		B	Feb. 20	Feb. 20	1.25	1	+	0	0	+	0	0	Do.
						2	+	0	0	+	0	0	Do.
		B	Mar. 25	Mar. 25	1.5	1	+	tr	0	+	tr	0	Do.
						2	+	tr	0	+	tr	0	Do.
			1915.	1915.									
		A	Dec. 30	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
			1916.	1916.									
		B	Jan. 23	Jan. 23	1.25	1	+	±	0	+	±	0	Do.
4550	...do					2	+	±	0	+	±	0	Do.
		B	Feb. 20	Feb. 20	1.25	1	+	0	0	+	0	0	Do.
						2	+	0	0	+	0	0	Do.
		B	Mar. 25	Mar. 25	1.5	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.

TABLE VIII.—*Wassermann reaction with glycerinated human sera, heated before having been mixed with the glycerin, kept in the cold storage.*

[One-tenth cubic centimeter of serum in each portion.]

No.	Secured.	Portion.	Heated.	Tested.	Amboceptor unit.	Reading.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.									
		A	Dec. 30	Dec. 30	1.0	1	+	+	tr	+	+	tr	Negative.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	±	0	+	±	0	Do.
4551	Dec. 29					2	+	±	0	+	±	0	Do.
		B	...do.....	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	...do.....	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
				1915.									
		A	...do.....	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	±	0	+	±	0	Do.
4552	...do.....					2	+	±	0	+	±	0	Do.
		B	...do.....	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	...do.....	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
				1915.									
		A	...do.....	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	±	0	+	±	0	Do.
4553	...do.....					2	+	±	0	+	±	0	Do.
		B	...do.....	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	...do.....	Mar. 25	1.25	1	+	±	0	+	±	0	Do.
						2	+	±	0	+	±	0	Do.
				1915.									
		A	...do.....	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	±	0	+	±	0	Do.
4554	...do.....					2	+	±	0	+	±	0	Do.
		B	...do.....	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	...do.....	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
				1915.									
		A	...do.....	Dec. 30	1.0	1	+	+	tr	+	+	tr	Do.
						2	+	+	tr	+	+	tr	Do.
				1916.									
		B	...do.....	Jan. 23	1.25	1	+	±	0	+	±	0	Do.
4555	...do.....					2	+	±	0	+	±	0	Do.
		B	...do.....	Feb. 20	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.
		B	...do.....	Mar. 25	1.25	1	+	+	0	+	+	0	Do.
						2	+	+	0	+	+	0	Do.

This volume serves a threefold purpose: It aims to give practitioners and students an account of our present knowledge of infection and immunity; to serve as a guide to the various immunologic methods for laboratory workers; and to outline a course for students.

The book is well illustrated with numerous half-tones and colored plates. It is divided into five parts: General Immunologic Technic; Principles of Infection; Principles of Immunity and Special Immunologic Technic; Applied Immunity in the Prophylaxis, Diagnosis, and Treatment of Disease—Specific Therapy; Experimental Infection and Immunity.

Part I gives clear and concise descriptions of laboratory apparatus and procedures which can easily be understood and followed by the reader.

Part II contains two chapters considering the various aspects of virulence and resistance, the various types of toxins, and the course of infection. Diphtheria toxin, tetanus toxin, snake venoms, aggressins, ptomains, and infection with animal parasites are discussed.

Part III deals with immunity and theories of immunity. The phagocytic theory of Metchnikoff and the side-chain theory of Ehrlich and different views regarding them are made clear to the reader. Bacterial vaccines, their preparation and use, antitoxins, and bactericidal sera are described. The Abderhalden test constitutes one of the most important chapters in this part of the book. Each constituent is separately discussed, and in eight brief paragraphs the test is made so clear that any student understands it.

Various antibodies, such as agglutinins, precipitins, cytolysins, bacteriolysins, and hæmolysins are adequately discussed in separate chapters. The Wassermann reaction with its various modifications and controls is freely discussed and adequately illustrated. Perhaps never before has the Wassermann reaction been made so clear. Kolmer makes the student understand the test. Complement-fixation tests for other diseases are amply dealt with.

Part IV is wholly practical in its nature. The reader is given a survey of prophylactic vaccination, vaccine and serum therapy, and chemotherapy.

Part V consists of sixty exercises in experimental infection and immunity. The book is closed with a well-prepared index.

From a practical point of view the price of the book may seem high for the average student, especially as these subjects ad-

vance so rapidly that the student is continually compelled to consult current periodical literature. The book is worth it and can be highly recommended to students and practitioners of medicine.

E. H. RUEDIGER.

Fever | its Thermotaxis and | Metabolism | by | Isaac Ott, A. M., M. D. | [11 lines] | [seal] | Paul B. Hoeber | 67–69 E. 59th Street | New York | 1914 | Cloth, pp. 1–166. Price, $1.50 net.

It is to be regretted that Professor Ott, who has contributed so much pioneer work on this subject, should appear as the author of the present booklet. This series of lectures seems to have been hastily written and very superficially edited. Professor Ott discusses particularly the heat centers, "thermotaxic nerves," heat dissipation and production, and metabolism on fever. The booklet is of little value either to the worker in the medical sciences or to the practitioner.

R. B. GIBSON.

Bacteriological Methods | in | Food and Drugs Laboratories | with an | introduction to micro-analytical methods | by | Albert Schneider, M. D., Ph. D. | [4 lines] | 87 illustrations | and 6 full page plates | Philadelphia | P. Blakiston's Son & Co. | 1012 Walnut Street | 1915 | Cloth, pp. i–viii+1–288. Price, $2.50 net.

Within recent years increasing attention has been given in Food and Drugs Laboratories to bacteriological methods. The chemical methods formerly used almost exclusively did not furnish all the data demanded by modern sanitary requirements. Accordingly bacteriologists have been engaged to assist in the enforcement of the food and drug laws. However, their activities were handicapped by lack of definite information and standards on the subject, the fragments of knowledge which they could use on these subjects being widely scattered. To supply this growing and urgent need for a suitable book on bacteriological methods in food and drug laboratories, Doctor Schneider undertook the work here reviewed.

He prefaces the bacteriological methods proper by giving the essentials of microanalytical work, often required of bacteriologists in food and drug laboratories. Under bacteriological methods he considers the various means used to detect the number and kind of microörganisms which may be present in foods and drugs in a normal, adulterated, or spoiled condition and the significance of their presence. The methods and technique given are such as have been used and tested before. Their collection in one volume is a great convenience to a worker

in this line. The standardization of disinfectants usually treated in an unsatisfactory manner in most bacteriologies is fully considered. A bibliography would add to the usefulness of the book. However, it is the best book of its kind of which we know, it is clearly written, is well printed and illustrated, and contains much useful information.

CHAS. E. GABEL.

Aids | to | Tropical Medicine | by | Gilbert E. Brooke | M. A. Cantab., L. R. C. P. Edin., D. P. H., F. R. G. S. | Port Health Officer, Singapore | [7 lines] | second (seal) edition | London | Baillière, Tindall & Cox | 8, Henrietta Street, Covent Garden | 1915 | Cloth, pp. i–xii+1–230. Thirty-seven text figures. Price cloth, 5/ net; paper, 3 net.

In this edition of "Aids to Tropical Medicine" by Brooke some new material has been added. The small size of the volume makes it convenient for use in the field. Like most books of this type omissions and errors have occurred in it. It is an open question whether books like this fill any real need in medical literature.

J. A. J.